

ON SOME FURTHER IMPROVEMENTS IN
THE PROCEDURES FOR TESTING AND
JUDGING BY THE NAKED EYE
OF THE
AGGLUTINATING AND BACTERIOLYTIC
EFFECTS EXERTED BY THE SERA
OF PATIENTS

*SUFFERING FROM, OR PREVENTIVELY INOCULATED
AGAINST, TYPHOID FEVER, MALTA FEVER,
AND TUBERCULOUS AFFECTIONS*

BY

A. E. WRIGHT, M.D. DUB.

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ON SOME FURTHER IMPROVEMENTS IN
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LYTIC EFFECTS EXERTED BY
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SUFFERING FROM, OR PREVENTIVELY INOCULATED AGAINST,
TYPHOID FEVER, MALTA FEVER, AND TUBERCULOUS
AFFECTIONS.

I PROPOSE to set forth in this communication a simplified and improved technique which has a practical application wherever we desire to add progressive dilutions of a fluid to another fluid. In particular—and this is the point which interests us here—the method has a useful practical application in connexion with the accurate quantitative determination of the effect exerted upon suspensions of bacteria and finely divided bacterial particles by agglutinating and bacteriolytic sera, and, I may add, salt solutions.

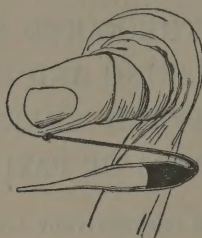
In the technique which is to be described the apparatus is narrowed down to a blood capsule, used for collecting the blood, and a single capillary tube,¹ a slip of glass, and a rubber teat used in the actual testing operations. The operations of progressively diluting the serum (or salt solution) and of mixing the successive dilutions in each case with the bacterial suspension are completed in uninterrupted sequence. Further, the effect exerted in the entire series of dilutions is submitted to the eye at a glance. The technique of the testing operation, essentially the same in all cases, may be described by setting forth the actual operations which would be undertaken in testing the blood of a tuberculous patient subjected to inoculation with the new

¹ At the moment of sending this to press I learn that Dr. B. W. Collingwood had already, in the course of work in South Africa, modified my original method of serum testing as is here done by the substitution of a single capillary tube for a series of such tubes.

tuberculin with a view to the development of protective tuberculotropic substances in his blood

Method of drawing off a sample of blood and obtaining the serum.—I have already elsewhere² described what I conceive to be the most convenient form of blood capsule and the most convenient procedure for collecting blood. This procedure is briefly as follows. A capsule such as that depicted in Fig. 1

FIG. 1.



Showing method of taking blood from the finger.

is taken in hand. Its straight upper extremity is formed into a pricker by fusing it in the flame of a match and drawing it out (after withdrawing it from the flame) into a very fine spicule. After serving its purposes the sharp end is broken off in order to provide a channel for the air to escape when the capsule is filled in through the curved limb. The necessary blood is obtained by making pressure on the pulp of the finger after winding a bandage round the digit. When the finger pulp has been evacuated in this way the ligature is relaxed and reapplied and pressure is again made. This manoeuvre is repeated until a sufficient quantum of blood has been obtained. The straight upper limb of the capsule is then warmed in the flame and its extremity sealed. The contained air which has been rarefied by the heating now contracts and draws the blood back into the body of the capsule. The extremity of the bent limb thus left free is now sealed in its turn. The next step is to shake down the blood into the unoccupied upper end of the capsule. After a sufficient interval has been allowed for clotting the hand centrifuge is brought into use, the capsule being suspended by its bent limb into the tube of the apparatus. When the serum has been obtained by centrifugalisation the bent limb is snapped off with a bone forceps and the capsule is placed, as shown in Fig. 2, upright in a perforated rubber bung.

Preparation of the test fluid.—In the case where the response which has been made by a patient to inoculations of the new tuberculin is to be elicited the most convenient

² Proceedings of the Royal Society, 1902, Vol. lxxi.

test fluid to employ is a suspension of the extremely fine detritus obtained by grinding down cultures of the tubercle bacillus. Koch prescribes that this suspension should be made by triturating the tubercle powder in carbolised physiological salt solution. He incidentally refers to the fact that the test fluid becomes "hypersensitive" on keeping. This is equivalent to stating that with this fluid fallacies may arise by "spontaneous" agglutination and deposition. In my personal experience such "spontaneous" agglutination and deposition sooner or later occur in every suspension made in accordance with the directions of Koch. In some cases these phenomena manifest themselves already after 24 hours—i.e., in control tubes filled in with a perfectly freshly prepared suspension.

The train of ideas which I have elsewhere³ developed in setting forth a theory with regard to the intimate nature of agglutination led me to inquire whether the chloride of sodium in the test fluid was the active agent in calling forth these "spontaneous" agglutinations. I find that this is so. When all excess of salt is avoided by making up the

FIG. 2.



The capsule placed in the bung.

suspension of the tubercle powder with carbolised distilled water, and by employing to dilute the serum a 1 in 1000 solution of common salt, instead of the 8·5 per 1000 solution prescribed by Koch, fallacious agglutinations and precipitations are, in my experience, completely avoided.

The following modification of Koch's procedure furnishes a trustworthy test fluid. A minute quantity of the tubercle powder is placed in an agate mortar. Distilled water to which 0·5 per cent. of carbolic acid has been added is now poured upon the powder drop by drop. After triturating for a few minutes the turbid suspension thus obtained is syphoned into a capsule similar to that figured above in connexion with the collection of blood from the finger. After sealing the ends the capsule is centrifugalised for a few minutes in a hand centrifuge and the supernatant milky fluid is employed for the test.

Procedure for mixing the test fluid with progressive dilutions of the serum.—A capillary pipette is taken in the

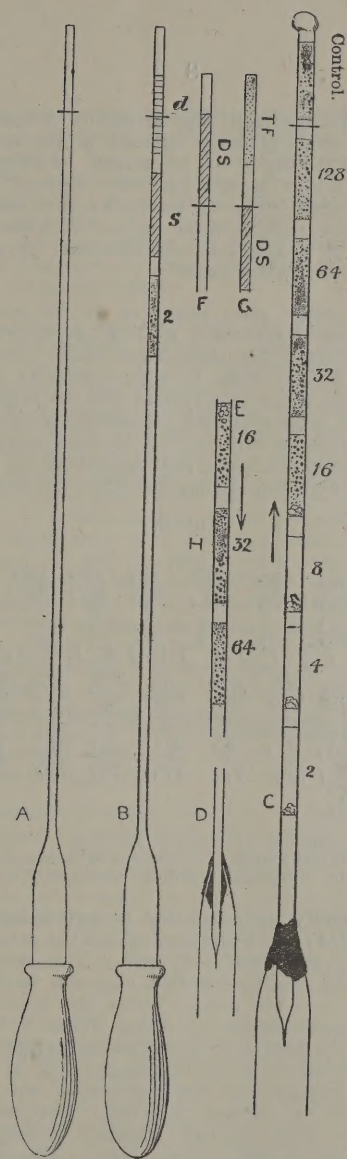
³ THE LANCET, May 9th, 1903, p. 1299.

hand, either such a simple pipette as that shown in Fig. 3, A and B, or, better, such a "throttled pipette" as is exhibited in Fig. 3, C and D. This last is made from the simple form of pipette by drawing out in the flame of a peep-light the capillary stem at the point where it comes off from the neck; breaking it across here; coating with sealing-wax the outside of the capillary stem near its throttled extremity; and then passing the capillary stem down through the truncated and heated butt end, originally the expanded portion of the simple pipette. A completely air-tight joint is formed in the neck of the tube by the solidification of the melted sealing-wax. By the throttling of the pipette we obtain a more effective control over the movements of the fluid in the tube and we avoid the free evaporation which in the case of the simple form of pipette occurs through the patent neck. When we bring the pipette into use we aspirate into the stem of the pipette one volume of the undiluted serum, a bubble of air to serve as an index, and one volume of the test fluid. We expel these and mix as indicated in Fig. 4 on the lower and left-hand corner of an ordinary microscopic slide. Of the mixture thus obtained we re-aspirate exactly one volume into the stem of our pipette.

Having divided off with a suitable bubble of air we now introduce into the stem of our pipette one volume of serum and one volume of the inert diluent (a 1 in 1000 dilution of chloride of sodium). The contents of the pipette are at this stage as shown in Fig. 3 B. We again expel the two last mentioned volumes, mixing on the upper left-hand corner of the slide as indicated in Fig. 4. The quantum of diluted serum thus obtained will provide our second test mixture and will in addition, if we consent to practise the little economies to which attention is directed in Fig. 3, F and G, furnish material for our successive dilutions. It will be seen that in F there has been filled into the stem of the pipette in lieu of a complete volume of diluted serum an incomplete volume eked out by a considerable bubble of air. In G there has been added a quantum of test fluid which—as will be recognised on considering that it is completed to one volume by the identical bubble of air—is exactly equal to the quantum of diluted serum which now occupies the portion of the tube above the fiduciary line. Having mixed on the slide in the situation indicated in the figure for this particular mixture we re-aspirate into the pipette as in the case of the former and all subsequent mixtures exactly one volume of the mixed fluids. We now make our next serum dilution, employing for this purpose a full volume of the twofold dilution of serum and, of course, a full volume of the inert diluent.

Proceeding upon these principles we fill in in succession into the stem of our pipette a series, ordinarily a series of seven or eight graduated dilutions of serum mixed in each case with a precisely equivalent volume of the bacterial

FIG. 3.

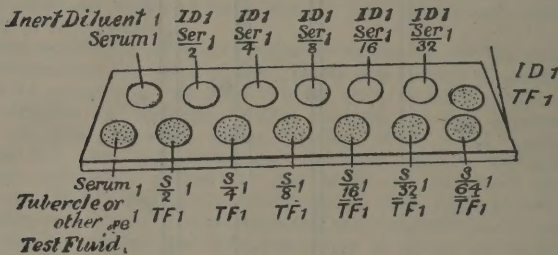


A, Simple pipette. B, Simple pipette containing : at *a*, one volume of serum mixed with one volume of test fluid ; at *s*, one volume of serum ; at *d*, one volume of diluting fluid. C, Throttled pipette in section. D, Throttled pipette filled in with progressive serum dilutions mixed in each case with an equal volume of test fluid. F and G, Showing method of economising in mixing diluted serum (Ds) in each case with an equal volume of test fluid (TF). H, Shows portion of the stem of pipette inverted in order to bring into view the character of the deposit.

suspension. We complete the series by introducing into the pipette a mixture of equal volumes of the inert diluent and test fluid to serve as a control. Finally we seal up the upper orifice of the capillary stem in the flame, inscribe the patient's name on the wide end of the tube, and place the pipette upright upon its base in a test-tube rack. We then leave it to stand in the incubator or, as the case may be, on the laboratory bench.

Phenomena which come into view in the successive test mixtures and the significance of these phenomena.—Before considering in detail the phenomena which come into view in a capillary pipette filled in with successive mixtures of the test fluid and serum of a patient who has responded with a production of tuberculotropic substances to inoculations of the new tuberculin it will be well to clear away a series of pre-suppositions in connexion with agglutination tests which are gradually incrusting themselves round our minds.

FIG. 4.



Progressive dilutions of serum (upper row of drops) and mixture of these progressive dilutions in each case with an equal volume of bacterial test fluid (lower row of drops).

1. It is currently laid down that an agglutination reaction may be *accepted* only in the case where the serum has been subjected to a considerable dilution. This statement holds good only if the running together of a few bacteria to form small clumps invisible except under the microscope is to rank as an agglutination reaction. What it really comes to is that if we accept as adequate an agglutination altogether inconspicuous to the naked eye and if we allow a considerable interval for its appearance we must exact a high degree of serum dilution. But reflection will make it clear that we can maintain exactly the same strictness in our standard if we relax in the matter of our dilutions and become correspondingly more exacting in other respects. Our exactions may, for instance, take the form of demanding that the agglutination effect shall manifest itself instantaneously, that the agglutination effect shall make

its appearance in a dense bacterial suspension, and that it shall be so pronounced as to discover itself to the naked eye. The advantages of the policy of working under conditions which involve a minimum dilution of the serum—and this is the policy followed out in the procedure described above—are very considerable. We dispense with all necessity for a microscope and we obtain our result with an absolute minimum of delay—in the case of an ordinary typhoid serum practically instantaneously.⁴

2. It is currently assumed that the effect which comes under observation when serum is mixed *in vitro* with a bacterial suspension is a pure agglutination effect. It is possible that little else comes under observation when the serum is highly diluted. But in low dilutions (certainly in the case where serum is mixed with a typhoid culture and, so far as I can judge, also in the case where serum is mixed with the tubercle test fluid) there is superadded to the agglutination effect also a macroscopically visible bacteriolytic effect.⁵ It follows that we cannot correctly interpret the phenomena which come into view in a mixture of serum and culture unless we bear in mind that bacteriolysis may be going on in each case hand in hand with agglutination. In particular we must bear in mind that an agglutination effect occurring in lower dilutions of serum is often after a certain interval completely masked by the dissolution of the clumps of agglutinated bacteria which were formed on first mixture. We must bear in mind also that a falling away of the bacteriolytic power such as occurs during the "negative phase" which succeeds upon a bacterial inoculation or upon a multiplication of bacteria in the infected organism, would tend to bring the agglutination reaction more into evidence.

These preliminary matters disposed of we may turn to the consideration of the phenomena which would manifest themselves in our capillary pipette in the case of a patient in whose blood tuberculotropic substances have been developed. After an interval of some 12 hours flocculation and deposition associated with a corresponding clarification of the supernatant fluid was observable in a certain number of the test mixtures. In Fig. 3, C, complete sedimentation is represented as having occurred in the two-, four-, and

⁴ I may note that such an instantaneous and macroscopically visible agglutination obtained in a mixture of equal parts of undiluted serum and typhoid culture is in my experience quite as conclusive of the diagnosis as an agglutination visible under the microscope after a quarter of an hour in a 1 in 50 dilution of the serum.

⁵ I have elsewhere (Brit. Med. Jour., April 4th, 1903) shown that the bacteriolytic effect exerted on the typhoid bacillus can be quantitatively determined by adding to the serum an enumerated culture of the typhoid bacillus and recounting it after digestion with the serum, conducting the enumeration in each case under the microscope by the method described by me in THE LANCET of July 5th, 1902. Normal human serum dissolves in a quarter of an hour at blood heat over 1,000,000,000 of the typhoid bacilli with which my experiments were carried out.

eight-fold dilutions of the serum.⁶ In the 16- and 32-fold dilutions a partial sedimentation has occurred. In the case of the higher dilutions of the serum and in the control the fluid is here shown as having remained evenly milky. A more minute study of the appearances brings out certain further points. The precipitate in the two-fold dilution of the serum is in most cases—presumably as a consequence of bacteriolysis—markedly less in bulk than the precipitate in the next following dilution. Be it remembered in this connexion that the pipette has been filled in with exactly equal quantities of each successive mixture. Again, after standing, in particular after long standing, a deposit makes its appearance also in the higher dilutions of the serum and in the control. This settling down can be distinguished from that which is causally connected with agglutination by two characteristics. In the first place it is associated with a clarification of these test mixtures which is in each case progressive from above downwards. (This point is not clearly brought out in the figure.) In the second place the deposit which is due to simple gravitation differs in character from that which is the result of agglutination. While the deposit in the latter case consists of conglomerated masses of bacterial particles irregularly piled one upon the other in such a way as to furnish an irregular surface outline (shown in Fig. 3, c, in connexion with the four- and eight-fold dilutions of the serum) the deposit in the former case consists of isolated bacterial particles forming a perfectly flat layer. Where, as illustrated in the figure in connexion with the 16- and 32-fold dilutions of the serum, attention to these characters does not entirely dispel doubt, a very simple device will reveal the character of the deposit. It is only necessary to invert the pipette for a few minutes and to bear in mind that a deposit composed of isolated bacterial particles will diffuse itself evenly through the fluid, while a deposit consisting of conglomerated bacterial particles will fall down in the form of separate pellets.

In a future communication I propose to draw attention to the importance of carrying out the method of blood examination in connexion with the inoculation of the new tuberculin.

Lower Seymour-street, W.

⁶ The agglutination—possibly by reason of the falling away of the bacteriolytic effect in the higher dilutions—generally manifests itself first in the eight-fold dilution of the serum.

